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APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
09/529,722	04/19/2000	DAVID J SQUIRRELL	1284-765	3155

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EXAMINER
STEADMAN, DAVID J

ART UNIT	PAPER NUMBER
2761	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)
	09/529,722	SQUIRRELL ET AL.
	Examiner	Art Unit
	David J. Steadman	1652

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 19-32 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 19-32 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☒ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Application Status

The request filed on 12/27/01 for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 09/529,722 is acceptable and a CPA has been established. An action on the CPA follows.

Claims 19-32 are pending in the application.

Oath/Declaration

1. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02. The oath or declaration is defective because: the application is objected to because of alterations which have not been initialed and/or dated as is required by 37 CFR 1.52(c). A properly executed oath or declaration which complies with 37 CFR 1.67(a) and identifies the application by application number and filing date is required.

Drawings

2. The drawings submitted with this application have not been reviewed by a draftsperson at this time. Upon allowance of the application, the draftsperson will perform a review. Direct any inquiries concerning drawing review to the Drawing Review Branch (703) 305-8404.

Specification/Informalities

3. The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed. The following title is suggested: "Method for Obtaining Luciferase Free of Thermosensitive Adenylate Kinase Catalytic Activity". See MPEP § 606.01.

Claim Objections

4. Claim 27 is objected to because of the following informalities: the term "according claim 25" is grammatically incorrect and should be replaced with, for example, "according to claim 25". Appropriate correction is required.

Claim Rejections - 35 USC § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 19-32 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

6. Claims 19 (claims 20-23 dependent therefrom) and 24 recite the limitations "the process" in claim 19, "the E. coli adenylate kinase" in claim 24, . There is insufficient antecedent basis for this limitation in the claim. It is suggested that the terms be replaced with "the method", E. coli adenylate kinase", repsectively.

7. Claim 30 (claim 32 dependent therefrom) recite the limitation "said second marker" in line 4 referring to "a selection marker" in line 3. There is insufficient antecedent basis for the limitation "said second marker" in the claim. It is suggested that the term "a selection marker" be replaced with, for example, "a second selection marker".

8. Claim 31 is confusing in the recitation of "said second selection marker" as there is no "second selection marker" defined in claim 29.

9. Claims 27 and 28 are confusing in the recitation of "comprises a prokaryotic cell" and "comprises a recombinant E. coli cell". It is unclear how a recombinant cell can comprise a prokaryotic or recombinant E. coli cell. It is suggested that the terms be replaced with, for example, "is a prokaryotic cell" and "is a recombinant E. coli cell", respectively.

10. Claims 29 (claim 31 dependent therefrom) and 30 (claim 32 dependent therefrom) are indefinite in the recitation of "adenylate kinase in a form which is denatured under given conditions". It is unclear

from the claim as written as to whether the "given conditions" are pH or temperature conditions as recited in claim 25 or some other "given conditions". It is suggested that applicants clarify the meaning of the claims.

11. Claim 29 (claims 30-32 dependent therefrom) is unclear in the recitation of "detecting those in which adenylate kinase is denatured". It is unclear as to whether the term "those" refers to transformants with a denatured adenylate kinase (AK) or to conditions which denature the AK. It is suggested that applicants clarify the meaning of the claim. For purposes of compact prosecution, the examiner has interpreted the term "those" as meaning transformants with a denatured AK.

12. Claim 25 (claims 26-32 dependent therefrom) is unclear in the recitation of "luciferase remains unaffected". It is unclear as to applicants' intended meaning of the term "unaffected". It is suggested that the term be replaced with, for example, "luciferase maintains at least partial enzymatic activity".

Claim Rejections - 35 USC § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

13. Claims 19-23 and 25-32 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 19, 20, 21 (claim 22 dependent therefrom), 23, 25 (claims 26-28 dependent therefrom), 29, and 30 (claims 31 and 32 dependent therefrom) are rejected because the claims recite a recombinant cell comprising a first polynucleotide encoding luciferase (Luc) and a polynucleotide encoding a genus of mutant AK polypeptides that are denatured under a genus of pH or temperature conditions, wherein the luciferase is unaffected, methods for producing said recombinant cell, and methods for using said

recombinant cell for producing luciferase. The specification teaches the structure of only one representative species of mutant AK polypeptides, namely, E. coli AK with mutations at positions 87 or 107 and is inctivated at temperatures of 40 °C or greater. The specification fails to disclose any other mutant AK polypeptides that are denatured at any given pH or temperature conditions under which luciferase remains stable by any identifying structural characteristics or properties other than the functionality of being mutant AK polypeptides that are denatured under pH or temperature conditions under which luciferase remains stable. Given the lack of description of additional representative species of mutant AK polypeptides that are denatured under pH or temperature conditions as encompassed by the genus of the claims, applicants have failed to sufficiently describe the claimed invention, in such full, clear, concise and exact terms that a skilled artisan would recognize that Applicants were in possession of the claimed invention.

14. Claims 19-23 and 25-32 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a recombinant cell comprising a polynucleotide encoding a Luc that is thermostable at temperatures of 37 °C or greater and a polynucleotide encoding a mutant AK polypeptide that is inactivated at temperatures of 37 °C or less, wherein the Luc maintains at least partial enzymatic activity and methods of making said recombinant cell or using said recombinant cell for the production of Luc, does not reasonably provide enablement for a recombinant cell comprising a first polynucleotide encoding *any* Luc that is stable under *any* pH or temperature conditions and a polynucleotide encoding *any* mutant AK polypeptide that is denatured under *any* pH or temperature conditions, wherein the Luc is unaffected, methods for producing said recombinant cell, and methods for using said recombinant cell for producing Luc. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re* Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the

presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 19, 20, 21 (claim 22 dependent therefrom), 23, 25 (claims 26-28 dependent therefrom), 29, and 30 (claims 31 and 32 dependent therefrom) are so broad as to encompass a recombinant cell comprising a first polynucleotide encoding *any* Luc that is stable under *any* pH or temperature conditions and a polynucleotide encoding *any* mutant AK polypeptide that is denatured under *any* pH or temperature conditions, wherein the Luc is unaffected, methods for producing said recombinant cell, and methods for using said recombinant cell for producing Luc. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of Luc polypeptides that are stable under *any* pH or temperature conditions and AK polypeptides that are inactivated under *any* pH or temperature conditions, wherein the Luc is unaffected as broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to a recombinant cell comprising a polynucleotide encoding a Luc that is thermostable at temperatures of 37 °C or greater and a polynucleotide encoding a mutant AK polypeptide that is inactivated at temperatures of 37 °C or less, wherein the Luc maintains at least partial enzymatic activity and methods of making said recombinant cell or using said recombinant cell for the production of Luc.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of

such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass a recombinant cell comprising a first polynucleotide encoding *any* Luc that is stable under *any* pH or temperature conditions and a polynucleotide encoding *any* mutant AK polypeptide that is denatured under *any* pH or temperature conditions, wherein the Luc is unaffected, methods for producing said recombinant cell, and methods for using said recombinant cell because neither the specification nor the prior art establish: (A) the predictability that a Luc polypeptide can be exposed to *any* pH or temperature conditions that inactivate AK activity, wherein the Luc remains unaffected as applicants have not demonstrated that Luc can be exposed to *any* pH or temperatures without loss of enzymatic activity; Kajiyama et al. (Biochemistry 32:13795-99) teach that even a thermostable mutant Luc undergoes some loss of activity at 50 °C over time (page 13796, Figure 2); (B) regions of the AK protein structure which may be modified with an expectation of AK enzymatic activity being susceptible to inactivation by *any* pH or temperature conditions, wherein the Luc maintains enzymatic activity; (C) the general tolerance of AK to modification and extent of such tolerance; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a recombinant cell comprising a first polynucleotide encoding *any* Luc that is stable under *any* pH or temperature conditions and a polynucleotide encoding *any* mutant AK polypeptide that is denatured under *any* pH or temperature conditions, wherein the Luc is unaffected, methods for producing said recombinant cell, and methods for using said recombinant cell for producing Luc. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re* Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological

characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re* Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

15. Claims 19-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over EP 373962 in view of Belinga et al. (J Chromat A 695:33-40), Gilles (Proc Natl Acad Sci, USA 83:5798-5802), and Kajiyama et al. (Biochemistry 32:13795-13799). Claims 19-32 are drawn to a recombinant cell comprising a first polynucleotide encoding Luc that is stable under pH or temperature conditions and a polynucleotide encoding a mutant AK polypeptide that is denatured under pH or temperature conditions or temperature conditions, wherein the Luc is unaffected, methods for producing said recombinant cell, and methods for using said recombinant cell for producing Luc.

EP 373962 teaches that a thermostable enzyme can be purified from unwanted contaminants that interfere with the intended use of the thermostable enzyme (column 2, lines 38-43) by engineering host cells, including procaryotes such as E. coli (column 4, lines 11-12), to produce a desired enzyme and the desired enzyme can be purified from contaminating proteins by denaturation at a temperature that does not denature the desired thermostable enzyme (column 2, lines 45-49).

Belinga et al. teach that the presence of AK interferes with Luc bioluminescence assays by producing light with nucleotides other than ATP and disclose that it is necessary to remove AK during the purification of luciferase (page 33).

Kajiyama et al. teach a vector encoding a mutant thermostable Luciola cruciata luciferase (abstract). The mutation resulting in the thermostable luciferase is a substitution of threonine with

isoleucine at position 217 (abstract). Kajiyama et al. teach that the encoded wild-type L. cruciata Luc was inactivated by incubation at 50 °C for 40 min, while the thermostable Luc maintained over 30 % enzymatic activity (page 13796, left column).

Gilles et al. teach thermosensitive mutants of E. coli with a mutation in the endogenous adk gene encoding AK (page 5798, left column). Characterization of the mutant AK encoded by the endogenous E. coli gene revealed the presence of a substitution of serine for proline at position 87 (page 5798, right column). Gilles et al. teach the thermosensitive AK is inactivated by incubation of crude extracts at 40 °C (page 5798, left column).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of EP 373962, Belinga et al., Kajiyama et al., and Gilles et al. for an E. coli with a gene encoding a thermosensitive AK, wherein the gene is endogenous or introduced via homologous recombination, and transformed with an expression vector encoding a thermostable Luc and culturing the transformant to express the thermostable Luc followed by cell lysis and purification of the encoded thermostable Luc from the thermosensitive AK by heat treatment as taught by EP373962. One would have been motivated for an E. coli with a gene encoding a thermosensitive AK transformed with an expression vector encoding a thermostable Luc and culturing the transformant to express the thermostable Luc followed by cell lysis and purification of the encoded thermostable Luc from the thermosensitive AK by heat treatment as taught by EP 373962 in order to remove the contaminating AK as taught by Belinga et al. as described above. One would have a reasonable expectation of success for an E. coli with a gene encoding a thermosensitive AK transformed with an expression vector encoding a thermostable Luc and culturing the transformant to express the thermostable Luc followed by cell lysis and purification of the encoded thermostable Luc from the thermosensitive AK by heat treatment because of the results of EP 373962, Kajiyama et al., and Gilles et al. Therefore, claims 19-32, drawn to a recombinant cell comprising a first polynucleotide encoding Luc that is stable under pH or temperature conditions and a polynucleotide encoding a mutant AK polypeptide that is denatured under pH or temperature conditions or temperature

conditions, wherein the Luc is unaffected, methods for producing said recombinant cell, and methods for using said recombinant cell for producing Luc would have been obvious to one of ordinary skill in the art.

This rejection has been reinstated, having been previously set forth in Paper No. 4 and subsequently withdrawn in Paper No. 6. Applicants traversed the rejection in Paper No. 5 on the grounds that the examiner required no less than five references for a showing of prima facie obviousness, thus providing evidence to the contrary. In response to applicant's argument that the examiner has combined an excessive number of references, reliance on a large number of references in a rejection does not, without more, weigh against the obviousness of the claimed invention. See *In re Gorman*, 933 F.2d 982, 18 USPQ2d 1885 (Fed. Cir. 1991).

Applicants argue that the method of EP 323962 (Backman et al.) describes a crude approach to the purification of proteins that have exceptional stability but that luciferase in no way has exceptional stability compared to the proteins of Backman. In response to applicant's argument, it is noted that neither the degree of luciferase thermal stability nor the "crudeness" of the method are recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. Furthermore, the ordinarily skilled artisan would have been well aware from the disclosure of Backman that it is not the degree of stability of the desired protein that is critical, but that there exists conditions under which the desired protein is stable and the contaminating protein(s) are not.

Applicants argue that EP 323962 provides no suggestion to modify the host cells, other than for expression of the thermostable enzyme. As disclosed by Gilles et al., no modification is necessitated as mutant E. coli strains with genes encoding thermolabile AKs exist. In view of the disclosures of Belinga and Backman, one of ordinary skill in the art would have recognized that these mutant E. coli would be appropriate host cells for the expression of thermostable Luc as they would allow the purification of Luc from AK by a simple heat denaturation step. Furthermore, one of ordinary skill in the art would recognize that *any* E. coli could be modified to produce a thermolabile AK by introducing a gene encoding a thermolabile AK into any E. coli strain by homologous recombination.

Applicants further argue that one of ordinary skill in the art would not apply the method of EP 323962 (Backman et al.) to the thermostable Luc of Kajiyama et al. because such thermostable Lucs as taught by Kajiyama et al. are "not in the same league of thermostability as the enzymes of Backman". Applicants' argument is not found persuasive because, based on the teachings of Kajiyama et al., one of ordinary skill in the art would not have applied the temperatures used by Backman for the purification of thermostable Luc. One of ordinary skill in the art would have recognized that such temperatures would inactivate the thermostable Luc and would have instead applied a temperature as demonstrated by Kajiyama that would allow the retention of Luc activity.

Applicants argue that Belinga et al. teach a method of purifying luciferase from adenylate kinase by a chromatographic procedure, thereby teaching away from the instant invention. Applicants' argument is not found persuasive. One of ordinary skill in the art would have recognized that Belinga et al. do not teach away from the method of purifying enzymes by heat denaturation as taught by EP 323962 and applied to the purification of Luc from AK, as the method of EP 323962 would have required less time, fewer steps, and required fewer reagents and materials to practice than the method of Belinga et al., thus providing motivation for one of ordinary skill in the art to practice the method of EP323962 rather the method of Belinga et al.

Applicants also argue that the examiner used impermissible hindsight in constructing the rejection by applying a mosaic of prior art. In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Applicants further present arguments based on the teachings of references not applied in the instant grounds of rejection (Liang et al. and Kiel et al.) and therefore, the examiner will not respond to these grounds of traversal.

Conclusion

16. No claim is in condition for allowance. All claims are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The Examiner can normally be reached Monday-Friday from 7:30 am to 2:00 pm and from 3:30 pm to 5:30 pm. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Group is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.

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GROUP 1800
1600